DIRECT EFFECTS OF OXYGENATED CRYSTALLOID OR BLOOD CARDIOPLEGIA ON ISOLATED MYOCYTE CONTRACTILE FUNCTION

John R. Handy, Jr., MD[a]
B. Hugh Dorman, MD, PhD[b]
Martyn J. Cavallo, MD[b]
Robert B. Hinton, BA[a]
Raymond C. Roy, MD, PhD[b]
Fred A. Crawford, MD[a]
Francis G. Spinale, MD, PhD[a]

The majority of myocardial protective techniques performed in the United States incorporate hypothermic, hyperkalemic blood or crystalloid cardioplegia. Oxygenated blood cardioplegia has not been compared with oxygenated crystalloid cardioplegia in an isolated myocyte model of hypothermic, hyperkalemic cardioplegic arrest in which direct measurements of contractile function and myocyte swelling can be made. Accordingly, isolated myocyte contractile function and myocyte profile surface area were examined after hypothermic arrest with oxygenated crystalloid or blood cardioplegia. ***Methods:*** **Isolated left ventricular pig myocytes were randomly assigned to undergo cardioplegic arrest for 2 hours at 4° C. Either oxygenated crystalloid or blood cardioplegia was used. After 2 hours, myocytes were reperfused with standard cell medium at 37° C and contractile function was examined. A control group of myocytes was maintained in cell medium at 37° C for 2 hours. Myocyte velocity of shortening (micrometers per second) was examined at baseline and after β-adrenergic stimulation (isoproterenol, 25 nmol/L). Velocity of shortening declined equally from baseline control values (65 ± 2 μm/sec) in the groups subjected to oxygenated crystalloid cardioplegia and blood cardioplegia (37 ± 2 μm/sec and 42 ± 1 μm/sec, respectively; $p < 0.05$).** ***Results:*** **Although β-adrenergic stimulation caused a significant increase in velocity of shortening in all myocyte groups, the increase was less pronounced in myocytes subjected to crystalloid cardioplegia (157 ± 6 μm/sec) and blood cardioplegia (159 ± 6 μm/sec) than in normothermic control myocytes (205 ± 7 μm/sec; $p < 0.05$). Myocyte profile surface area, an index of cell volume, was measured in all myocyte groups. Myocyte surface area increased equally after cardioplegic arrest and rewarming in both cardioplegia groups (crystalloid 4119 ± 53 μm^2; blood 3924 ± 48 μm^2); surface areas in both cardioplegia groups were significantly greater than in the normothermic control group (3158 ± 39 μm^2, $p < 0.05$).** ***Conclusion:*** **Equivalent effects of oxygenated crystalloid and blood cardioplegia were observed with respect to myocyte contractile function, inotropic responsiveness, and intracellular volume regulatory processes.**
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From the Department of Surgery[a] and Department of Anesthesiology and Perioperative Medicine,[b] Medical University of South Carolina, Charleston, S.C.

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Address for reprints: Francis G. Spinale, MD, PhD, Division of Cardiothoracic Surgery, Medical University of South Carolina, 171 Ashley Ave., Charleston, SC 29425.

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Cardioplegic arrest during cardiac operations is typically accomplished by hyperkalemic solutions that cause rapid myocyte membrane depolarization and thereby uncouple excitation and contraction. Despite significant improvements in the delivery and composition of cardioplegic solutions over the past decade, left ventricular (LV) dysfunction and myocardial edema continue to complicate the postoperative course of some patients having cardiac operations.[1, 2] Although a number of different myocardial preservation techniques in current practice limit ischemic injury during cardiac surgical procedures, the optimal cardioprotective strategy is still unclear. Research efforts have recently focused on compari-

sons between blood and oxygenated crystalloid cardioplegic solutions. Randomized clinical studies and experimental preparations in vivo that have compared blood and oxygenated crystalloid cardioplegic solutions have yielded mixed results with regard to the preservation of contractile function and myocardial edema. Proponents of blood cardioplegia claim superior protection from ischemic and reperfusion damage. They note improved ventricular function and reduced edema owing to enhanced oxygen-carrying capacity, endogenous free radical scavenging, and improved onconicity, buffering, and rheologic effects.[3] Moreover, more than 70% of cardiothoracic surgeons currently use blood cardioplegia.[4] Other studies, however, have shown oxygenated crystalloid cardioplegia to be comparable with blood cardioplegia or even superior in preserving contractile function at lower temperatures.[5-7]

The changes in LV loading conditions, heart rate, and plasma catecholamine concentrations that occur after cardioplegic arrest and rewarming make it difficult to determine direct effects of various cardioplegic solutions on LV contractile function in clinical or whole-heart preparation studies. Examination of contractile properties of isolated myocytes has certain distinct advantages over studies of in vivo preparations, including removal of loading conditions, absence of neurohormonal influences, and the capacity to directly examine contractile function without confounding effects from populations of nonmyocyte cells or alterations in coronary perfusion. Furthermore, the extracellular milieu can be precisely controlled so that specific and direct influences of various cardioplegic solutions on contractile function can be determined. Efforts to preserve contractile function and reduce myocardial edema after cardioplegic arrest have become increasingly important as the proportion of older, debilitated patients with more compromised LV function in the cardiac surgical population has increased.[8] Accordingly, the present study compares the direct effects of oxygenated crystalloid and blood cardioplegia on contractile function and volume regulation in an isolated myocyte model of hypothermic, hyperkalemic cardioplegic arrest to better understand optimal cardioprotective strategies.

Methods

Myocyte isolation. Yorkshire swine (25 to 30 kg) were the source of myocytes for this study. All animals were treated and cared for in accordance with the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985). Myocyte isolation was performed as previously described.[9] After the animal was anesthetized with 2% isoflurane in oxygen, a sternotomy was performed and the heart quickly extirpated and placed in oxygenated Krebs solution. The great vessels were rapidly removed at the aortic and pulmonary valves, and a region of the LV free wall incorporating the circumflex artery (5 by 5 cm) was excised and used for myocyte isolation. The left circumflex artery was cannulated, the distal branches were ligated, and the tissue was rinsed free of blood with 35 ml of a modified Kraft-Bruhe solution (KCl, 80 mmol/L; K_2HPO_4, 30 mmol/L; $MgSO_4$, 5 mmol/L; glucose, 10 mmol/L; Na_2–adenosine triphosphate, 5 mmol/L; taurine, 20 mmol/L; creatine, 5 mmol/L; succinate, 5 mmol/L; and HEPES buffer, 5 mmol/L) supplemented with nitrilotriacetic acid (5 mmol/L) and 0.1% salt-free bovine serum albumin. Collagenase (0.5 mg/ml, Worthington type II; 146 U/mg) was then added to 75 ml of the modified Kraft-Bruhe solution and the tissue was perfused with the collagenase solution for 35 minutes. All perfusion procedures were performed at 37° C and perfusion solutions were continuously aerated with 95% oxygen and 5% carbon dioxide. The tissue was then minced into 2 mm sections and added to an oxygenated titration solution of Kraft-Bruhe solution containing 2% bovine serum albumin, deoxyribonuclease II (DNase, 51 Kunitz units per milliliter, type IV, Sigma Chemical Company, St. Louis, Mo.), $CaCl_2$ (300 μmol/L), and collagenase (0.5 mg/ml). The tissue and titration solution were transferred to a centrifuge tube and gently agitated. After 15 minutes, the supernatant was removed and filtered and the cells were allowed to settle. The myocyte pellet was then resuspended in Dulbecco's modified Eagle's medium (Nutrient Mixture F-12, pH 7.4, Ca_2 2.0 mmol/L, Gibco Laboratories, Grand Island, N.Y.). The number of viable myocytes was counted at 100× magnification with a hemocytometer (Reichert-Jung, Leica, Inc., Optical Products Div., Buffalo, N.Y.) and resuspended to a final concentration of 5×10^4 cells/ml. We[9] have previously shown that viable myocytes exclude trypan blue, maintain a rod shape, are calcium tolerant, respond to electrical stimulation, and are stable in culture.

Myocyte contractile function and experimental design. An aliquot (2 ml) of the isolated myocyte suspension was placed on coverslips previously coated with a laminin/fibronectin matrix (Matrigel, Collaborative Biomedical, Inc., Bedford, Mass.) and incubated at 37° C for 1 hour. The myocytes were then placed in a thermostatically controlled chamber (37° C) and fitted for a coverslip for imaging on an inverted microscope (Axiovert IM35, Carl Zeiss, Oberkochen, Germany). The volume of the chamber was 2.5 ml, and it contained two stimulating platinum electrodes and a miniature thermocouple (CN7100; Omega Engineering, Inc., Stamford, Conn.). The medium within the chamber was oxygenated before entering a miniature pump system (733100 Reglo; Ismatec, Switzerland), which changed the medium within the chamber every 15 minutes. The myocytes were imaged with a 20× long-working distance Hoffmann Modulation Contrast objective (Modulation Optics, Inc., Greenvale, N.Y.) with

a final magnification of 1100×. Myocyte contractions were elicited by field stimulating the tissue chamber at 1 Hz (S11, Grass Instrument Co., West Warwick, R.I.) by means of current pulses of 5 msec duration and voltages 10% above contraction threshold.[9] The polarity of the stimulating electrodes was alternated at every pulse to prevent the buildup of electrochemical by-products. Myocyte contractions were imaged with a charge-coupled device with a noninterlaced scan rate of 240 Hz (GPCD60, Panasonic Communications and Systems Div., Secaucus, N.J.). Myocyte motion signals were captured with the cell parallel to the video raster lines, and this video signal was input through an edge detector system (Crescent Electronics, Sandy, Utah). The changes in light intensity at the myocyte edges were used to track myocyte motion.[9] The distance between the left and right myocyte edges was converted into a voltage signal, digitized, and input to a computer (80286; ZBV2526, Zenith Data Systems, St. Joseph, Mich.) for subsequent analysis.

Stimulated myocytes were allowed a 5-minute stabilization period after electrical stimulation, and contraction data for each myocyte were recorded from a minimum of 20 consecutive contractions. Parameters computed from the contraction profiles included myocyte resting length (micrometers), velocity of shortening (micrometers per second), and velocity of relengthening (micrometers per second). Myocyte percent shortening was determined as the percentage difference between maximum and minimum cell length for each contraction. Myocyte velocity computations were obtained by differentiating the digitized contraction profiles. These parameters were calculated for each contraction and the results were averaged for 20 contractions. Digital data regarding myocyte length throughout contraction were graphically plotted on a time scale to make sequential analog contraction profiles.

After collection of baseline myocyte contractile performance, the myocytes were then randomly assigned to one of the following treatment protocols: *normothermic control*—incubation in cell culture medium at 37° C for 2 hours ($n = 150$); *crystalloid cardioplegia*—incubation with an oxygenated crystalloid cardioplegic solution (K^+ 24 mEq, oxygen tension 300 ± 25 mm Hg, pH 7.38 ± 0.05, $n = 140$) and stored at 4° C for 2 hours; *blood cardioplegia*—incubation with an oxygenated blood cardioplegic solution (80% tromethamine-buffered blood, hematocrit value 17% to 22%, K^+ 22 mEq, oxygen tension 280 ± 20 mm Hg, pH 7.52 ± 0.03, $n = 140$) and stored at 4° C for 2 hours. Because the blood cardioplegia protocol required continuous gentle agitation throughout the hypothermic incubation period to prevent erythrocytes from becoming attached to the laminin/fibronectin matrix and thus obscuring the myocytes, both cardioplegia protocols included continuous gentle agitation of the myocytes throughout the hypothermic incubation period. After treatment, the myocytes were transferred to the stimulation chamber and rewarmed with oxygenated medium until the bath temperature returned to 37° C. Steady state contractile measurements were obtained as described in the previous section. To define β-adrenergic responsiveness, contractile function of the myocytes from the three treatment protocols was then measured in isoproterenol, 25 mmol/L.

Isolated myocyte surface area. To determine the differential effects of the individual cardioplegic solutions on myocyte volume regulatory processes, isolated profile surface area was measured in myocytes subjected to the three treatment protocols outlined in the previous section. It has been previously demonstrated that profile surface area directly reflects cell volume.[10] Isolated myocyte surface area was measured by means of methods previously described by this laboratory.[9] In brief, after normothermic incubation or cardioplegic arrest and rewarming, myocytes were immediately placed in a buffered sodium cacodylate solution containing 2% paraformaldehyde and 2% glutaraldehyde (pH 7.4, 325 mOsm/L). Preserved myocytes were examined on an inverted light microscope (IM-35, Carl Zeiss). Myocytes were imaged at 1000× magnification with an epifluorescence illuminator having a rhodamine filter. Isolated myocyte borders were digitized by means of an image analysis system (Zeiss/Kontron, IBAS), and profile surface area was computed by planimetry directly from the digitized image.

Data analysis. Changes in indices of myocyte contractile function between the control, crystalloid, and blood cardioplegia groups were examined by multivariate analysis of variance. If the analysis of variance revealed significant differences, pairwise tests of individual group means were compared by means of Tukey's procedure. All statistical analysis was performed with the use of standard statistical software programs (BMDP Statistical Software Inc., Los Angeles, Calif.). Results are presented as mean ± standard error of the mean. Values of $p < 0.05$ were considered to be statistically significant.

Results

Myocyte contractile function. Steady state myocyte contractile function and representative myocyte contraction profiles for normothermic control, oxygenated crystalloid cardioplegia, and blood cardioplegia groups are summarized in Table I and Fig. 1. In agreement with prior reports, myocyte contractile function after 2 hours of normothermic incubation was similar to baseline values obtained immediately after myocyte isolation.[11] Therefore only contractile function parameters after normothermic incubation are reported. Contractile function of myocytes exposed to either crystalloid cardioplegia or blood cardioplegia followed by rewarming was significantly worse than that of control myocytes. Specifically, the velocity of shortening was reduced 43% in the crystalloid cardioplegia group and 35% in the blood cardioplegia group compared with control values. Percent shortening was reduced by 42% and 33% in the crystalloid and blood cardioplegia groups, respectively. The velocity of relengthening, which is an index of active relaxation, was also significantly reduced by more than 40% in both cardioplegia groups. Thus, consistent with past studies, hypothermic, hyperkalemic cardioplegic arrest caused a re-

duction in isolated myocyte contractile process.[9, 11] Moreover, cardioplegic arrest with both oxygenated crystalloid and blood cardioplegic solutions decreased myocyte contractile function to a similar degree after rewarming.

Myocyte β-adrenergic responsiveness. Isoproterenol (25 nmol/L) was added to normothermic control, crystalloid cardioplegia, and blood cardioplegia myocytes to compare the effects of crystalloid and blood cardioplegia on β-adrenergic responsiveness (see Table I). Measurements of myocyte contractile function in the presence of isoproterenol for the three treatment groups are also provided in Table I. Isoproterenol caused a significant increase in myocyte contractile function in normothermic control, crystalloid cardioplegia, and blood cardioplegia myocytes. Specifically, the velocity of shortening and percent shortening were increased by greater than 100% over baseline values in all groups. However, contractile function indices of myocytes in either the crystalloid cardioplegia or blood cardioplegia groups after isoproterenol administration remained significantly lower than these indices for normothermic control myocytes (Table I). Oxygenated crystalloid cardioplegia and blood cardioplegia reduced β-adrenergic responsiveness equally.

Myocyte profile surface area. To determine changes in myocyte volumes after cardioplegic arrest with oxygenated crystalloid or blood cardioplegia, we measured myocyte profile surface area in normothermic control, crystalloid cardioplegia, and blood cardioplegia myocytes. As illustrated in Fig. 2, myocyte profile surface area formed a Gaussian distribution in normothermic control myocytes with a mean value of 3158 ± 39 μm^2. Crystalloid and blood cardioplegia resulted in a similar and significant increase in myocyte profile surface area to 4119 ± 53 μm^2 and 3924 ± 48 μm^2, respectively ($p < 0.05$ vs control).

Table I

	Baseline	*Isoproterenol (25 nmol/L)*
Percent shortening (%)		
Normothermic control	5.2 ± 0.1	11.2 ± 0.2†
Blood cardioplegia	3.5 ± 0.1*	9.6 ± 0.3*†
Crystalloid cardioplegia	3.0 ± 0.1*	9.6 ± 0.2*†
Velocity of shortening (μm/sec)		
Normothermic control	65 ± 2	205 ± 7†
Blood cardioplegia	42 ± 1*	159 ± 6*†
Crystalloid cardioplegia	37 ± 2*	157 ± 6*†
Velocity of relengthening (μm/sec)		
Normothermic control	70 ± 3	197 ± 8†
Blood cardioplegia	40 ± 2*	145 ± 7*†
Crystalloid cardioplegia	36 ± 2*	145 ± 6*†
No. of myocytes		
Normothermic control	150	132
Blood cardioplegia	140	119
Crystalloid cardioplegia	140	123

*$p < 0.05$ versus normothermic control.
†Versus baseline.

Discussion

Improved myocardial preservation during cardiac operations has remained a concern of cardiac surgeons for decades. Despite significant improvement in cardioplegic techniques, transient LV dysfunction and myocardial edema still occur after cardioplegic arrest and rewarming. The composition and delivery methods of cardioplegic solutions have undergone extensive investigation to minimize this LV dysfunction.[12] Recently, blood has been proposed as the most efficacious delivery vehicle for hyperkalemic cardioplegia to limit ischemic damage to myocardium.[12-14] The development of oxygenated crystalloid cardioplegic solutions also appears to represent a significant advance in cardiac protection.[5, 6, 15, 16] The ability of these cardioplegic solutions to preserve contractile function and volume regulatory processes has not been compared in isolated myocytes. In the present study, isolated myocyte contractile function and myocyte volume regulation were examined after hypothermic, hyperkalemic cardioplegic arrest with oxygenated crystalloid and blood cardioplegic solutions. The unique and significant findings of the present study were as follows: (1) both oxygenated crystalloid cardioplegia and blood cardioplegia caused a similar reduction in myocyte contractile function and β-adrenergic responsiveness and (2) both oxygenated crystalloid and blood cardioplegia resulted in a similar disruption of myocyte volume regulation.

Blood cardioplegia emerged in the 1980s, immediately gained popularity, and is currently the most widely used mode of cardioplegia.[4] Proponents of blood cardioplegia cite several theoretical advantages of the blood vehicle: (1) a reduction in systemic hemodilution, (2) improved onconicity and buffering because of the presence of blood proteins, (3) rheologic benefits on the microvasculature, (4) oxygen-derived free radical scavenging resulting from superoxide dismutase and catalase, and (5) improved oxygen-carrying capacity.[12, 17] Such potential benefits would be expected to reduce myocardial damage during cardioplegic arrest and thereby result in improved preservation of contractile function

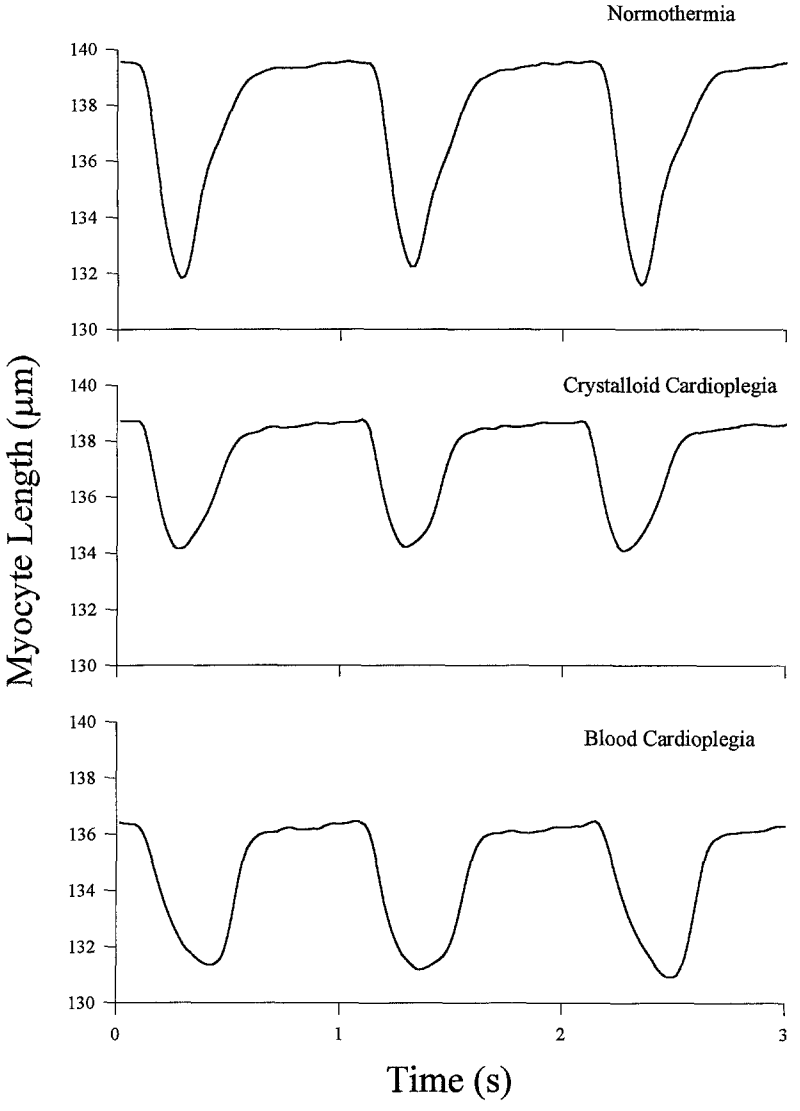

Fig. 1. Representative contractile profiles of normothermic (control) myocytes and myocytes exposed to hypothermic cardioplegic arrest with either oxygenated crystalloid or blood cardioplegia. Myocyte contractile profiles were measured after rewarming during field stimulation at 1 Hz. A significant and equal depression in the extent of shortening was observed after hypothermic cardioplegic arrest with both oxygenated crystalloid cardioplegia and blood cardioplegia. Please see Table I for summary results.

after rewarming. However, experimental and clinical studies comparing the preservation of contractile function after cardioplegic arrest between blood cardioplegia and oxygenated crystalloid cardioplegia have yielded mixed, equivocal results. Both cardioplegic solutions result in excellent cardiac protection and appear to preserve contractile function better than nonoxygenated solutions.[14-16, 18, 19] At infusion temperatures of 20° C or greater or with reperfusion of acutely ischemic myocardium, blood cardioplegia appears to provide superior results.[20] However, at lower infusion temperatures blood cardioplegia and oxygenated crystalloid cardioplegia appear to provide similar preservation of contractile function and recovery of high-energy phosphates, provided that a low-sodium crystalloid solution is not used.[5, 15, 16] Moreover, at 4° C, oxygenated crystalloid cardioplegia has been shown to result in improved recovery of ventricular function as determined by end-systolic pressure-length relationships.[6] In the current study, there was no difference in the preservation of contractile function between isolated myocytes after cardioplegic arrest with blood or oxygenated crystalloid cardioplegia. The

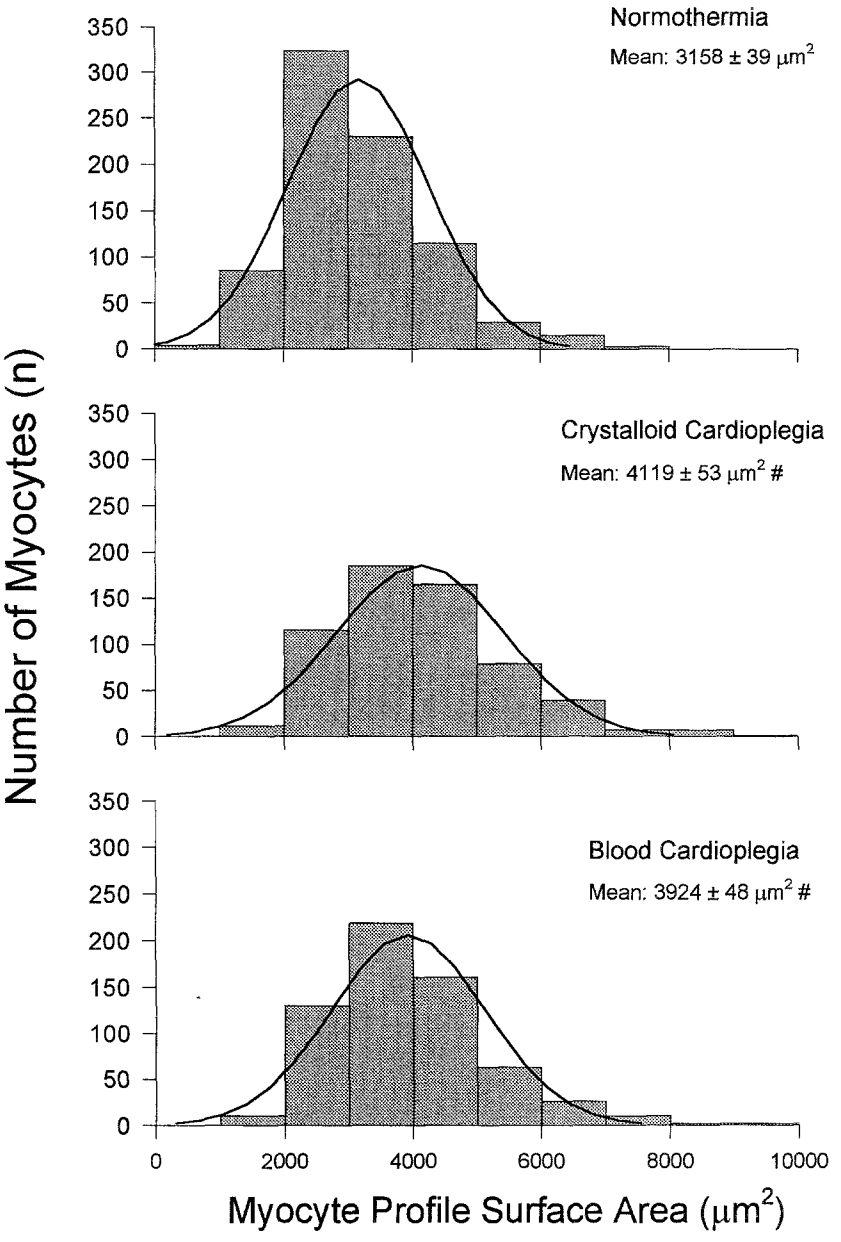

Fig. 2. Myocyte profile surface area for normothermic (control) myocytes and for myocytes after hypothermic cardioplegic arrest with oxygenated crystalloid cardioplegia and blood cardioplegia. The number of myocytes measured is indicated on the *Y axis*. Myocyte profile surface area formed a Gaussian distribution. A significant and equal increase in profile surface area was observed for myocytes that underwent hypothermic cardioplegic arrest and rewarming with oxygenated crystalloid cardioplegia and blood cardioplegia. Please see *Results* for summary statistics.

reduction in myocyte contractile function after cardioplegic arrest and rewarming was similar in the two experimental groups. This reduction in myocyte contractile function after cardioplegic arrest and rewarming provides a cellular basis for the reduced LV function observed clinically and in isolated heart preparations after hyperkalemic cardioplegic arrest. It further suggests that at 4° C, there are no advantages to blood cardioplegia relative to oxygenated crystalloid cardioplegia in the preservation of isolated myocyte contractile function. However, incubation temperatures at 4° C for cardioplegic arrest would tend to favor oxygenated crystalloid cardioplegic solutions. Magovern and colleagues[21]

demonstrated better preservation of isovolumic developed LV pressure with blood cardioplegia than with crystalloid cardioplegia at 20° C, but at 10° C or 4° C crystalloid solutions provided better myocardial protection. Progressive hypothermia shifts the oxygen-hemoglobin dissociation curve to the left, which reduces release of oxygen from blood cardioplegic solutions. In contrast, all of the oxygen carried by a crystalloid solution is available for release because of its linear oxygen dissociation curve.[15] Moreover, the solubility of oxygen in crystalloid solutions increases as the temperature decreases. At 10° C, therefore, an oxygenated crystalloid cardioplegic solution has more oxygen available for release than arterial whole blood, oxygenated pump perfusate, or an oxygenated blood cardioplegic solution.[22] Future studies that more carefully examine temperature-dependent differences in cardioplegic techniques on myocyte contractile function would be appropriate. A frequent consequence of hypothermic, hyperkalemic cardioplegic arrest is myocardial edema.[2, 23] Myocardial water content significantly increases after cardioplegic arrest and reperfusion; this increase is directly associated with a reduction in LV compliance and contributes to a subsequent deterioration in LV performance.[2, 24] Moreover, myocardial edema may limit coronary perfusion during reoxygenation.[25] In the current study, hyperkalemic cardioplegic arrest with both oxygenated crystalloid cardioplegia and blood cardioplegia resulted in similar and significant increases in isolated myocyte profile surface area. Because surface area is directly proportional to myocyte volume, the increase in myocyte profile surface area after cardioplegic arrest represents cellular swelling.[10] These results suggest that similar alterations in myocyte volume regulatory processes occur after hyperkalemic cardioplegic arrest with either oxygenated crystalloid or oxygenated blood cardioplegia. Prior reports have also shown no significant difference in the extent of myocardial edema after cardioplegic arrest with either blood or crystalloid cardioplegia in experimental heart preparations.[26, 27] A significant increase in myocardial water content, however, has been shown after cardioplegic arrest with nonoxygenated cardioplegic solutions compared with oxygenated cardioplegic solutions, irrespective of the cardioplegic vehicle used. This observation further confirms the critical importance of oxygen in cardioplegia.[15, 18] Oxygenation of cardioplegic solutions may preserve high-energy phosphates, which can limit acute changes in myocardial capillary membrane integrity that may contribute to increases in myocardial edema.[18] Moreover, the extracellular hyperkalemia associated with either blood or crystalloid cardioplegic solution causes prolonged myocyte membrane depolarization and associated changes in ionic balance including sodium influx and an increase in intracellular calcium by sodium-calcium exchange, increased calcium release from sarcoplasmic reticulum, and an influx of calcium through slow calcium channels.[28] Such alterations in ionic homeostasis also appear to play a role in the observed abnormalities in volume regulation with reperfusion and rewarming, especially since improved control of calcium transients during cardioplegic arrest appears to attenuate myocyte swelling.[9] Thus myocardial edema may be primarily related to the hyperkalemia-induced membrane depolarization and may not be a direct consequence of the cardioplegia vehicle.

β-Adrenergic stimulation in the present study caused an increase in myocyte contractile function in normothermic control myocytes and in myocytes after hyperkalemic cardioplegic arrest. The contractile function response to β-adrenergic treatment, however, was blunted to a similar degree after cardioplegic arrest with both oxygenated crystalloid cardioplegia and oxygenated blood cardioplegia. Such reduced β-adrenergic responsiveness with either cardioplegic solution may have clinical implications, because treatment with β-adrenergic agents is frequently necessary after prolonged hyperkalemic cardioplegic arrest. The β-receptor uncoupling with subsequent dampened adenylyl cyclase activity that has been shown after hyperkalemic cardioplegic arrest probably contributes to the reduced β-adrenergic responsiveness observed.[29] Thus blood cardioplegia did not appear to be better than oxygenated crystalloid cardioplegia in enhancing the capacity of the myocyte to respond to an inotropic stimulus.

This study examined contractile function after cardioplegic arrest with blood cardioplegia and oxygenated crystalloid cardioplegia in an isolated myocyte model of hypothermic, hyperkalemic cardioplegic arrest. An isolated myocyte model used in the present study allows for direct measurements of contractile function without changes in loading conditions, neurohormonal activity, or alterations in coronary perfusion that could alter ventricular performance in vivo. Although the isolated myocyte model has some advantages over an in vivo preparation, it has important limitations as well. An

isolated myocyte system allows for maximal solute diffusion capacity between the cytosol and extracellular space. This differs from an in vivo preparation in which capillary diffusion distances are affected by coronary artery disease and hypertrophy, and uniform temperature control and maintenance are not achieved. Furthermore, cardioplegia affects the entire myocardium, which includes not only myocytes, but also endothelium, fibrocytes, and the interstitium. It is possible that a beneficial effect of blood cardioplegia on myocardial protection occurs at a site removed from the myocyte. For example, a recent in vivo canine model of cardiopulmonary bypass demonstrated superior protection of the microvascular endothelium by blood when compared with oxygenated crystalloid cardioplegia.[30]

In summary, oxygenated crystalloid cardioplegia and oxygenated blood cardioplegia were equivalent in the preservation of contractile function and β-adrenergic responsiveness in an isolated myocyte model of hypothermic, hyperkalemic cardioplegic arrest. Moreover, disruption of myocyte volume regulation was similar after hyperkalemic cardioplegic arrest with either blood or oxygenated crystalloid cardioplegia. This suggests that the effects of blood cardioplegia and oxygenated crystalloid cardioplegia are equivalent with respect to contractile processes and volume regulation on the fundamental unit of the heart, the myocyte. If improved protection of myocyte contractile processes exits, it must be operative on other elements and mechanisms within the myocardium.

REFERENCES

1. Roberts AJ, Spies SM, Sanders JH, Moran JM, Wilkinson CJ, Lichtenthal PR, et al. Serial assessment of left ventricular performance following coronary artery bypass grafting. J Thorac Cardiovasc Surg 1981;81:69-84.
2. Weng ZC, Nicolosi AC, Detwiler PW, Hsu DT, Schierman SW, Goldstein AH, et al. Effects of crystalloid, blood, and University of Wisconsin perfusates on weight, water content, and left ventricular compliance in an edema-prone isolated porcine heart model. J Thorac Cardiovasc Surg 1992;103:504-13.
3. Krukenkamp IB, Silverman NA, Levitsky S. The effects of cardioplegic oxygenation on the correlation between the linearized Frank-Starling relationship and myocardial energetics in the ejecting post-ischemic heart. Circulation 1987; 76(Pt 5):122-8.
4. Robinson LA, Schwarz GD, Goddard DB, Fleming WH, Galbraith TA. Myocardial protection for acquired heart disease surgery: results of a national survey. Ann Thorac Surg 1995;59:361-72.
5. Rousou JA, Engelman RM, Breyer RH, Otani H, Lemeshow S, Das DK. The effect of temperature and hematocrit level of oxygenated cardioplegic solutions on myocardial preservation. J Thorac Cardiovasc Surg 1988;95:625-30.
6. Coetzee A, Roussouw G, Fourie P, Lochner A. Preservation of myocardial function and biochemistry after blood and oxygenated crystalloid cardioplegia during cardiac arrest. Ann Thorac Surg 1990;50:230-7.
7. Heitmiller RF, Deboer LW, Geffin GA, Toal KW, Fallow JT, Drop LJ, et al. Myocardial recovery after hypothermic arrest: a comparison of oxygenated crystalloid to blood cardioplegia. Circulation 1985;72(Suppl):II241-53.
8. Weintraub WS, Wenger NK, Jones EL, Craver JM, Guyton RA. Changing clinical characteristics of coronary surgery patients. Circulation 1993;88(Suppl):II79-86.
9. Cavallo MJ, Dorman BH, Spinale FG, Roy RC. Myocyte contractile responsiveness following hypothermic hyperkalemic cardioplegic arrest: disparity between exogenous calcium and β-adrenergic stimulation. Anesthesiology 1995;82:926-39.
10. Drewnowska K, Baumgarten CM. Regulation of cellular volume in rabbit ventricular myocytes. Am J Physiol 1991; 260:C122-31.
11. Dorman BH, Cavallo MJ, Hinton RB, Roy RC, Spinale FG. Preservation of myocyte contractile function following hypothermic hyperkalemic cardioplegic arrest with 2,3-butanedione monoxime. J Thorac Cardiovasc Surg 1996; 111:621-9.
12. Barner HB. Blood cardioplegia: a review and comparison with crystalloid cardioplegia. Ann Thorac Surg 1991;52:1354-67.
13. Roberts AJ, Moran J, Sanders JH, Spies SM, Lichtenthal PR, Kaplan KJ, et al. Clinical evaluation of the relative effectiveness of multi-dose crystalloid and cold blood potassium cardioplegia in coronary artery bypass graft surgery: a non-randomized matched-pair analysis. Ann Thorac Surg 1982;33:421-33.
14. Loop FD, Higgins TL, Panda R, Pearce G, Estafanous FG. Myocardial protection during cardiac operations. J Thorac Cardiovasc Surg 1992;104:608-18.
15. Bodenhamer RM, DeBoer LW, Geffin GA, O'Keefe DD, Fallon JT, Aretz TH, et al. Enhanced myocardial protection during ischemic arrest. J Thorac Cardiovasc Surg 1983;85: 769-80.
16. Guyton RA, Dorsey LMA, Craver JM, Bone DK, Jones EL, Murphy DA, et al. Improved myocardial recovery after cardioplegic arrest with an oxygenated crystalloid solution. J Thorac Cardiovasc Surg 1985;89:877-87.
17. Buckberg GD. Myocardial protection: an overview. Semin Thorac Cardiovasc Surg 1993;5:98-106.
18. Tabayashi K, McKeown PP, Miyamoto M, Luedtke AE, Thomas R, Allen MD, et al. Ischemic myocardial protection: comparison of nonoxygenated crystalloid, oxygenated crystalloid, and oxygenated fluorocarbon cardioplegic solutions. J Thorac Cardiovasc Surg 1988;95:239-46.
19. Engelman RM, Rousou JH, Lemeshow S, Dobbs WA. The metabolic consequences of blood and crystalloid cardioplegia. Circulation 1981;64(Suppl):II67-74.
20. Brown WM, Jay JL, Gott JP, Huang AH, Pan-Chih, Horsley WS, et al. Warm blood cardioplegia: superior protection after acute myocardial ischemia. Ann Thorac Surg 1993;55: 32-42.
21. Magovern GJ, Flaherty JT, Gott VL, Buckley BH, Gardner TJ. Failure of blood cardioplegia to protect myocardium at lower temperatures. Circulation 1982;66:60-7.

22. Digerness SB, Vanini V, Wideman FE. In vitro comparison of oxygen availability from asanguineous and sanguineous cardioplegia media. Circulation 1981;64(Suppl):II80-8.
23. Foglia RP, Steed DL, Follette DM, Deland E, Buckberg GD. Iatrogenic myocardial edema with potassium cardioplegia. J Thorac Cardiovasc Surg 1979;78:217-22.
24. Bolli R. Mechanism of myocardial "stunning." Circulation 1990;82:723-38.
25. Willerson JT, Watson JT, Hutton I, Templeton GH, Fixler DE. Reduced myocardial reflow and increased coronary vascular resistance following prolonged myocardial ischemia in the dog. Circ Res 1975;36:771-81.
26. Swanson DK, Myerowitz PD. Distribution of adenylates, water, potassium and sodium within the normal and hypertrophied canine heart following two hours of preservation. J Surg Res 1982;32:515-25.
27. Takamoto S, Levine FH, LaRaia PJ, Adzik NS, Fallon JT, Austen WG, et al. Comparison of single-dose and multiple dose crystalloid and blood potassium cardioplegia during prolonged hypothermic aortic occlusion. J Thorac Cardiovasc Surg 1980;79:19-28.
28. Grinwald PM. Calcium uptake during post-ischemic reperfusion in the isolated rat heart: influence of extracellular sodium. J Mol Cell Cardiol 1982;14:359-65.
29. Schwinn DA, Leone BJ, Spahn DR, Chesnut LC, Page SO, McRae RL, et al. Desensitization of myocardial β-adrenergic receptors during cardiopulmonary bypass: evidence for early uncoupling and late downregulation. Circulation 1991;84: 2559-67.
30. Murphy CO, Pan-Chih, Gott JP, Guyton RA. Microvascular reactivity after crystalloid, cold blood, and warm blood cardioplegic arrest. Ann Thorac Surg 1995;60:1021-7.